Human Galanin Modulates Human Colonic Motility in Vitro

Characterization of Structural Requirements

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Background: Human galanin (hGal) is a 30-residue non-amidated gut–brain peptide that shows considerable sequence divergence compared with galanin (Gal) forms of other species. Conflicting results have been reported with regard to the structural requirements for its modulatory action on gut motility. *Methods:* We investigated the effect of human and rat Gal and substituted analogues of Gal on the contractility of longitudinal muscle strips of the human colon in vitro. *Results:* Both hGal and rGal contracted the preparations in a concentration-dependent and tetrodotoxin-resistant manner without difference in sensitivity. The NH_2-terminally truncated peptides hGal (3–30) and rGal (3–29) were inactive, whereas the NH_2-terminal fragments, hGal (1–21) and rGal (1–18), remained fully responsive. Single amino acid substitutions at NH_2-terminal positions showed divergent results: substitution of Trp^2 reduced significantly potency and efficacy, whereas substitutions at positions 1, 3, 4, or 5 did not markedly modify the bioactivity of Gal. Galantide, a high-affinity Gal antagonist in the central nervous system, is a full agonist in human colonic smooth muscle. *Conclusion:* The COOH-terminal part of Gal contributes mainly the receptor-binding affinity of the peptide, whereas the NH_2-terminal region is essential for biologic activity.

Key words: Galanin analogues; galanin receptor; galantide; human colonic motility; human galanin; rat galanin

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Galanin (Gal), a 29-amino-acid peptide isolated from porcine small intestine (1), has subsequently been found in the gut of several species, including man. Recently, the primary structure of human galanin (hGal) has been identified (2–4). Compared with the 29-residue porcine (pGal), rat (rGal), and bovine galanin (bGal), hGal comprises 30 amino acids with an additional non-amidated serine residue at the COOH-terminus. hGal differs in 4, 6, or 7 amino acids from rGal, pGal, or bGal, respectively. All amino acid substitutions are restricted to the COOH-terminal part of the molecule (positions 16–30; see Table 1).

Galanin has numerous effects both in the central nervous system and in peripheral tissues (5–7). In various species it causes contraction or relaxation of gastrointestinal smooth muscle or modulates the action of other peptides or neurotransmitters on gut motor function. It may therefore be important for the regulation of gastrointestinal motility (5, 7, 8). However, the precise role of hGal, particularly in human tissues, is still unknown. We therefore investigated the effects of rGal and hGal and several Gal analogues on isolated human colonic strips.

MATERIALS AND METHODS

Materials and drugs

hGal, rGal, and their analogues and fragments were synthesized by solid-phase N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) strategy (2, 9). Peptides were purified by high-performance liquid chromatography (HPLC) to apparent homogeneity and characterized by mass spectrometry and amino acid analysis (2, 9). The purity of the peptides was greater than 95%. Galantide (also named M-15) was purchased from Saxon Biochemicals (Hannover, Germany); all other substances were bought from Sigma (Munich, Germany).

Preparation of human longitudinal colonic muscle strips

Segments of apparently macroscopically normal tissues from the ascending, transverse, descending, or sigmoid colon were taken from patients of both sexes (17 men and 15 women; age, 45 to 83 years) who underwent abdominal surgery for tumors of the colon. None of the patients had insulin-dependent diabetes mellitus or inflammatory bowel

Table 1. Primary structures of porcine, rat, human, and bovine galanin

	1	5	10	15	16	17	18	20	23	25	26	29	30
Galanin (porcine):	Gly	-Trp -Thr -Leu	-Asn -Ser -Ala	-Gly -Tyr -Leu	-Leu -Gly	-Pro -His	-Ala	-Ile	-Asp -Asn	-His -Arg	-Ser -Phe	-His -Asp -Lys	-Tyr -Gly -Leu -Ala
Galanin (rat):	...	... ...	... ...	... ...	... ...	... ...	...	Val	-Gly ...	... ...	... ...	Ser	Thr ... Thr -Ser
Galanin (human):	...	... ...	... ...	... ...	... ...	... ...	...	Leu	... Ser	... ...	... ...	Gln	His ... ...
Galanin (bovine):	...	... ...	... ...	... ...	... ...	... ...	...	Leu	... Ser	... ...	... ...	His	... ...

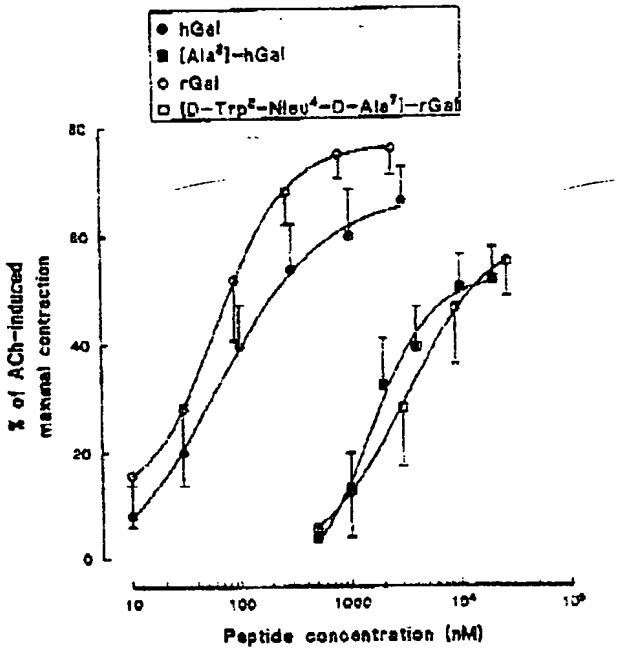

Fig. 1. Dose-response curves for the contractile effect of hGal, rGal, [Ala²]-hGal, and [D-Trp²-Nleu⁴-D-Ala⁷]-rGal on human colonic strips. Data are expressed as percentage of the maximal response obtained with acetylcholine (ACh) (100 μM). Each point is the mean of four to seven experiments; vertical bars indicate SEM.

disease. The patients were anesthetized with intravenous analgesia (fentanyl, midazolam) in combination with halothane or enflurane and N_2O/O_2. Full-thickness muscle strips from the taenia (length, 2.5 cm; width, 0.2–0.25 cm) were cut in parallel to the longitudinal axis and carefully freed from the mucosa.

Measurement of muscular activity

Tissues were used within 1 h after removal from the patients or after storage up to 44 h at 4°C in Krebs solution. The strips were suspended under a load of 1 g in organ baths filled with 3 ml Krebs solution bubbled with 95% O_2/5% CO_2 and maintained at 37°C. The composition of the Krebs solution was as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.18 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4, and 5.6 mM glucose. Responses were measured isometrically with Grass FT03 force displacement transducers connected to a Grass multichannel polygraph (model 79D). The muscle strips were allowed to equilibrate for 60–120 min. Experiments were started when the contractile response to acetylcholine (ACh) (1–10 μM) was constant. Only one concentration-response curve was obtained from each muscle strip. Doses of peptide were applied individually with a minimum of 20 min between each application.

Table II. Efficacies and potencies of hGal, rGal and their fragments and analogues on contraction of human colonic longitudinal strips

Peptide	Efficacy (%)	Potency (EC_{50}) (nM)	Relative efficacy (%)	Relative potency (%)	No. of experiments (n)
hGal	67 ± 6	62 ± 13	100	100	4
[Ala^1]-hGal	64 ± 8	49 ± 25	96	127	6
[Ala^2]-hGal	52 ± 6*	1445 ± 420*	78	4	7
[Ala^3]-hGal	61 ± 8	361 ± 62*	91	17	5
[Ala^4]-hGal	69 ± 11	39 ± 16	103	159	7
[Ala^5]-hGal	62 ± 10	113 ± 21	93	55	4
hGal (1–21)	74 ± 5	48 ± 20	110	129	6
hGal-NH_2	60 ± 11	85 ± 29	90	73	6
rGal	76 ± 5	63 ± 2	113	98	4
[$Nleu^4$]-rGal	66 ± 6	53 ± 2	99	117	4
[D-Trp^2-$Nleu^4$-D-Trp^7]rGal	55 ± 6*	3148 ± 327*	82	2	4
rGal (1–18)	66 ± 11	92 ± 13	99	67	4

Data are given as means ± SEM of n experiments. Efficacy is calculated as follows: (maximal effect of peptide/maximal effect produced by acetylcholine (100 μM)) × 100. Potencies were calculated from computer-fitted curves using the Fig. P. program. Relative potency and efficacy are expressed as percentage compared with hGal. Asterisks indicate statistical significance ($p < 0.05$) compared with hGal or rGal, respectively.

Data analysis

The contractile effect of all peptides tested was expressed as percentage of the maximal response obtained with ACh (100 μM). The concentration-response curves were analyzed, and the potency (EC_{50}) calculated from computer-fitted curves using the Fig. P program (Biosoft, Elsevier, Cambridge, UK).

Statistical analysis

Results are expressed as means ± SEM of n experiments. Statistical evaluation for significance was performed with the appropriate Student's t test for paired or unpaired data adapted for multiple comparisons in accordance with Bonferroni (10).

RESULTS

hGal (10–3000 nM) and rGal (10–2430 nM) contracted human colonic strips in a concentration-dependent manner and showed approximately the same potency and efficacy (Fig. 1, Table II). The maximal contraction obtained with hGal and rGal was 67 ± 6% and 76 ± 5%, respectively, compared with that of ACh (100 μM). The NH_2-terminally truncated derivatives hGal (3–30) and rGal (3–29) had no effect at concentrations up to 10 μM. The dose-response curves of the Gal analogues [Ala^1]-hGal, [Ala^3]-hGal, [Ala^4]-hGal, [Ala^5]-hGal, [$Nleu^4$]-rGal, and the fragments hGal (1–21) and rGal (1–18) were not markedly different from those of hGal and rGal (Figs. 2–4). The bioactivity of hGal

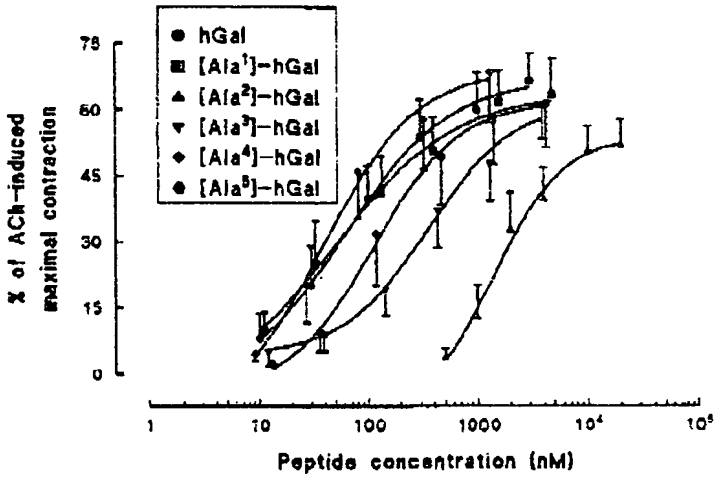

Fig. 2. Concentration-response curves for hGal and NH_2-terminally alanine-substituted hGal analogues. Results are expressed as percentage of the maximal response induced by acetylcholine (ACh) (100 μM). Each point is the mean of four to seven individual determinations, and vertical bars show SEM.

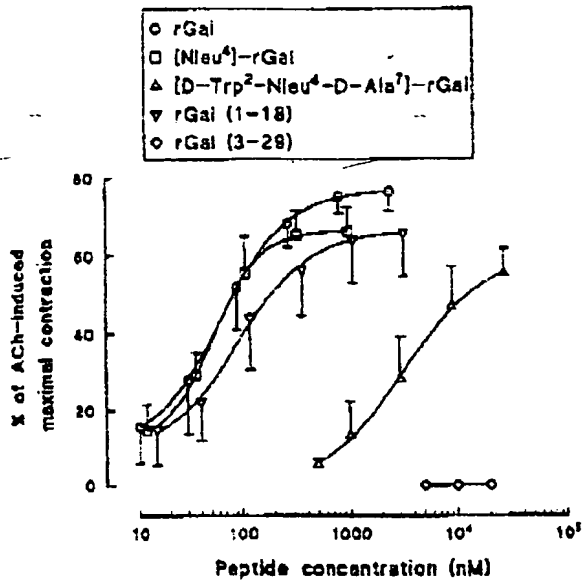

Fig. 3. Dose-response curves of the contractile effect of rGal and some rGal analogues and fragments on human colonic strips. Results are expressed as percentage of the maximal response induced by acetylcholine (ACh) (100 μM). Each point is the mean of four experiments: vertical bars show SEM.

was not significantly modified after COOH-terminal amidation (Fig. 4, Table II). In contrast, replacement of Trp^2 by Ala^2 showed a 23-fold loss in potency (Figs. 1 and 2, Table II). Simultaneous replacement of the three NH_2-terminally located amino acids in positions 2, 4, and 7 by [D-Trp^2-$Nleu^4$-D-Ala^7]-rGal as indicated showed the strongest decrease in potency (about 50-fold) (Table II). With the exception of [Ala^2]-hGal and [D-Trp^2-$Nleu^4$-D-Ala^7]-rGal, the peptides investigated had nearly the same efficacy (Table II). All potencies and efficacies are summarized in Table II.

The contractions induced by hGal and rGal were resistant to tetrodotoxin (TTX) (1 μM, $n = 4$ for each) and atropine (1 μM, $n = 4$ for each). To study the antagonistic potency of the putative Gal antagonist galantide (the chimeric peptide galanin (1–13)-substance P(5–11)), hGal (1 μM) was tested in the presence and absence of galantide (100 nM, 500 nM, and 1 μM). Surprisingly, galantide was found to contract human longitudinal colonic strips dose-dependently at the concentrations used. When the contraction induced by galantide had decreased to base line, a second application of hGal showed an unchanged responsiveness (102 ± 4; 104 ± 5; and 112 ± 13, $n = 8$) for each dose of galantide (Fig. 5).

DISCUSSION

The present study demonstrates that hGal is a potent modulator of human colonic motility in vitro. In comparison with rGal, no marked difference exists in the ability to contract human colonic longitudinal strips. The stimulatory effect of both peptides is TTX-resistant and probably mediated through the same receptor located on the surface of the smooth-muscle cell. Similar direct myogenic contractile effects of pGal have been described in smooth-muscle strips of human small intestine (11), human colon (12, 13), and human appendix (14). The concentration range of

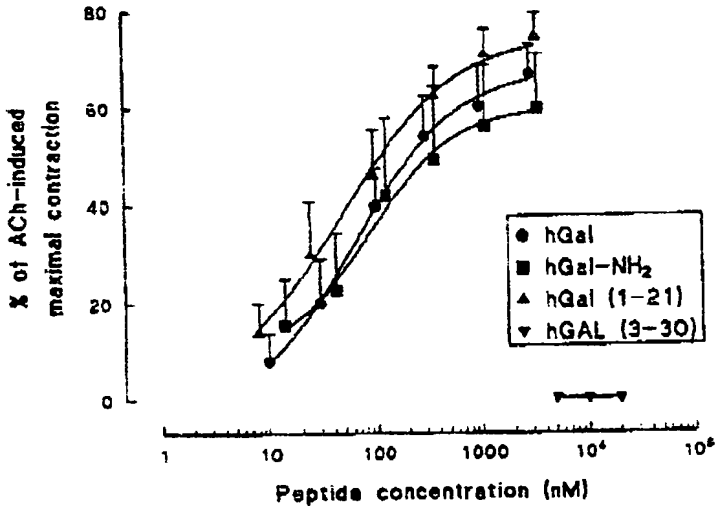

Fig. 4. Concentration-response curves for the contractile effect of hGal, hGal (1–21), and COOH-terminally amidated hGal on human colonic strips. Data are expressed as percentage of the maximal response induced by acetylcholine (ACh) (100 μM). Each point represents the mean of four to six experiments; vertical bars indicate SEM.

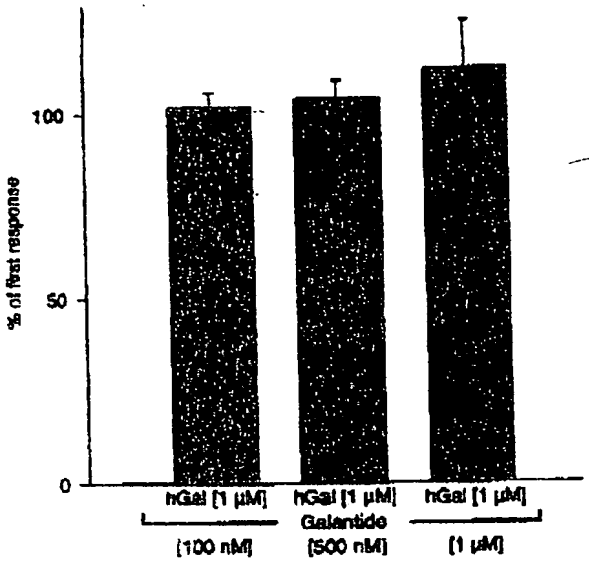

Fig. 5. Contraction of human colonic strips induced by hGal (1 μM) in the presence of increased concentrations of galantide. Values are means ± SE of eight experiments.

effective doses reported in these studies was nearly identical to that of hGal observed in this study. Therefore, the hGal receptor present in the gastrointestinal tract did not distinguish between the different molecular forms of Gal of various species. This might be explained by the fact that the species differences in the primary structures of Gal are exclusively restricted to the COOH-terminal part of the molecule (1–3; Table I). In agreement with our previous results obtained in the rat fundus with pGal and rGal (15), the present study clearly shows that the COOH-terminal region contributes mainly to the affinity of Gal towards its smooth-muscle receptor, whereas the NH_2-terminal region plays a critical role for its biologic activity. Particularly the first two amino acids (Gly^1-Trp^2) appear to be crucial for receptor activation.

There is experimental evidence from in vitro motility studies in the rat jejunum (16), guinea pig ileum (17), and taenia (18) supporting this hypothesis. Interestingly, Chakder & Rattan (19) reported that the COOH-terminal fragment Gal (15–29) caused an increase in resting tension of strips from the opossum internal anal sphincter, whereas native Gal produced a decrease in resting tone. A similar phenomenon has been observed in the isolated perfused rat pancreas, where the NH_2-terminal portion of Gal is essential for the inhibitory effect on glucose-induced insulin release (20), whereas short COOH-terminal fragments of the peptide conversely show insulinotropic effects (21). The presence of distinct receptor subtypes in the gastrointestinal tract of different species has been shown by using the NH_2-terminal fragment of Gal 1–10. This fragment induced similarly to native Gal contraction of jejunal strips of the rat but showed, in contrast to native Gal, no activity on neuromodulation of guinea pig taenia coli and rabbit iris sphincter (8).

Conflicting results with regard to the structural requirement of Gal for receptor activation have also been reported in other bioassay systems. The NH_2-terminal portion of Gal is crucial for inhibition of forskolin-induced cyclic adenosine 5'-monophosphate (AMP) production and insulin release from Rin m5F cells and inhibition of pentagastrin-stimulated gastric acid secretion in conscious rats, whereas COOH-terminal Gal fragments were inactive (22–24). In contrast, COOH-terminal fragments of Gal—that is, Gal (3–29) or Gal (9–29)—were able to inhibit cholecystokinin-8-stimulated amylase secretion (16).

These results support the view that Gal receptors are heterogeneous with regard to species and anatomic regions. Additionally, different receptor subtypes may be present in the same region, coupled to distinct effector systems, that might exert opposite effects.

Galantide has been shown to potently antagonize the action of Gal in the central nervous system (6, 25, 26) and the galanin-induced inhibition of the glucose-induced insulin secretion from mouse pancreatic islets (27). In the human colonic smooth muscle, however, galantide did not behave as a Gal antagonist. On the contrary, it acted as a full agonist. The same results were obtained using galantide on rat jejunal muscle strips and dispersed muscle cells from the guinea pig stomach (28). Surprisingly, galantide did not act as an antagonist of Gal on glucose-stimulated insulin release from the isolated perfused rat pancreas (29). Therefore, it seems that this chimeric Gal analog can behave both as an antagonist and as an agonist while interacting with different Gal receptor subtypes. Alternatively, Gal receptors in different species/tissues may have different agonist/antagonist ratios of activity with regard to this analog.

In summary, hGal is a modulator of human colonic motility. For the Gal receptor present in the human gastrointestinal tract, the COOH-terminal region of Gal contributes mainly to the affinity, whereas the NH_2-terminal region, in particular the first two amino acids (Gly-Trp), are crucial for receptor activation and biologic activity. COOH-terminal amidation, which is not present in native hGal, is not required for receptor interaction.

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